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Electron Microscopic Observation of Pulmonary Alveolar Structures

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Introduction

The structure of alveolus has long been one of the themes that attracted the attention of many physicians, and since 1808, when Reisseisen said that the air cells were merely extremities of as many bronchial tubes lined by ordinary mucous membrane, the covering tissue of the alveolar wall has become a pivotal point of discussions and many opinions have been expressed as to the existence and character of the covering tissue as well as the relations between the air spaces and the respiratory capillaries. It may be said, however, that we have not yet arrived at any definite conclusion.

The author of this article has, therefore, occupied himself in electron microscopic observation of the alveolar structure since July, 1954, for the purpose of contributing to the study of the pathologic physiology of the lung. Although the observation is still only half completed, some of the knowledge obtained so far about normal lungs is reported here.

I) Study of the Literature on Alveolar Structure

According to Akasaki, the various theories of alveolar structure, especially about the covering tissue of the alveolar wall, may be classified as follows:

1) The alveolar wall is completely covered with two kinds of respiratory epithelium: small nucleated cubic cells and larger non-nucleated plaques. (Eberth, Elenz, Kölliker, Ogawa etc.)

2) Respiratory epithelium is made up of small nucleated cubic cells, but not of non-nucleated plaques.

a) What has been considered a non-nucleated plaque is nothing but an extension of a nucleated epithelial cell. (Oppel, Briscoe, Aschoff etc.)

b) There are no non-nucleated plaques, and no continuous epithelial covering. (Akasaki, Seemann, Clara etc.)

3) All the nucleated cells situated on the alveolar wall are histiocytes or differentiated mesenchymal cells. (Lang, Poli-card, Testa etc.)

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4) Among the small nucleated cubic cells there are both epithelial cells and histiocytes.

- a) Non-nucleated plaques exist. (Kiyono, Majima, Foot etc.)
- b) Non-nucleated plaques are not mentioned.

In addition to these four groups classified by Akasaki, Miller and Hayek should be mentioned as representative scholars in the field of recent lung structure research. The former has recognized the existence of epithelial covering covered by nucleated epithelial cells and agreed with Kölliker's theory, although he has not specifically mentioned non-nucleated plaques, while the latter has denied the existence of non-nucleated plaques and acknowledged the existence of a certain kind of the nucleated epithelial cell. With regard to these cells, he says, there can be two functionally extreme cases, a) the pulmonary epithelial cell has a wing-shaped extension and covers the alveolar wall continuously, and b) the said extension is withdrawn, and the pulmonary epithelial cell becomes round, and the capillary and or the connective tissues are exposed between the round epithelial cells, to air in the alveolar space. Furthermore, he specified that there might also be a mid-way phenomenon between a) and b), and that sometimes, as in the case of b), the round epithelial cell would become a histiocyte.

All these results were observed with the light microscope and only two data have been reported so far from the United States of America by using electron microscopes, since this method has come to be used quite recently—one is the observation by F. N. Low in 1952 and 1953, and the other by R. H. Swigart and D. J. Kane in 1954. The former used human lungs to some extent, but he chiefly observed the lungs of rats, guinea-pigs, rabbits and dogs, while the latter observed rat lungs with the electron microscope. They have decidedly different opinions.

Their opinions will be considered below, but in brief, although they both are inclined to deny the existence of the non-nucleated plaques, Low asserts that the nucleated alveolar epithelial cells attenuate and extend themselves and wholly cover the alveolar wall, while Swigart and his colleague assert that there is septal material intervening between the capillary and pulmonary alveolus, and evidence for the existence of a continuous epithelial lining between the alveolus and capillary, described by Low, is lacking.

II) **Materias and Methods**

The chief material used by the present author was the human lung, but the lungs of mice, guinea-pigs and dogs were also observed. Wedge-shaped small pieces were excised from the normal lung tissue of adult pulmonary tuberculosis patients when they underwent pulmonary resection.

These pieces were rapidly fixed in 1% osmic acid according to Palade's method, and embedded in n-butylmethacrylate after the conventional washing and dehydration. Then these were sliced into ultrathin sections of from 1/18 micron to 1/22 micron thick,

and observed with the SM-K type electron-microscope.

An image observed by an electron-microscope may be said to show, so to speak, only a projection of a portion of the extremely thin tissue. But when observations are repeatedly carried out, the solid structure of the material can be inferred to a certain degree without using serial sections.

The following is a description of the results obtained by the author in regard to the human pulmonary alveoli and it may be added that in the cases of other laboratory mammals almost similar results were also perceived in general, except for a few details.

III) Observation and Discussion

A) *Covering Tissue of Alveolar Wall:*

Membranous covering tissues that completely and continuously cover the alveolar wall are invariable seen in every section when observed with an electron microscope as shown in figures 1-16. These membranous covering tissues are always observed to cover the alveolar walls completely, separating themselves clearly from the septal stomata, even when a portion of the alveolar structure is composed almost solely of capillaries (figs. 1 and 2), or when it is broader and includes connective tissue (figs. 3 and 4), or when it is composed of fibrous substances (fig. 5).

The two covering tissues are observed to be in contact, back to back, with each other at some places when the width of the alveolar structure is quite narrow (fig. 6) and are considered to face two adjacent air spaces respectively.

The thickness of this membranous alveolar covering tissue is not always even. A comparatively clear line which is called a basement membrane is always observed at the base, and the structure of the portion between the alveolar lining and the basement membrane is obviously cytoplasmic, because the structure is perceived to contain mitochondria here and there without containing fibrous material.

It may be said that the membranous covering tissue is epithelial, judging from the fact that it fulfils the two conditions that 1) it continuously and completely covers the surface of the alveolar wall and that 2) it is not mixed with other tissues, but is separated from them. Moreover, the fact that the covering tissue is always observed in any section shows that it is continuously connected with the bronchial covering tissue.

The stratum of the epithelial covering tissue is, in fact, extremely thin and Low has mentioned that its thickness averages 0.2 micron or less in the human lung, and that as it is difficult to observe, it seems to be absent in some locations. The continuity of this epithelial covering tissue is always noticeable in the human lung, however, when a well-cut section is carefully observed.

Then what is this epithelial covering tissue? To answer this question, from among various kinds of sections let us choose and observe the cell which is situated on the alveolar wall and is obviously considered to be nucleated alveolar epithelium, judging

from the cross-section of the nucleus.

Figs. 7, 8 and 15 show that the cytoplasm of the nucleated alveolar epithelium which is on the basement membrane and faces an air space, thins abruptly on both sides of the cellbody into a long extension with the same structure, and covers a capillary or the surface of connective tissue. This can be seen in all nucleated alveolar epithelial cells and the cell body itself is never seen as round or cubic without cytoplasmic extensions. It is to be expected, however, that there are some places seen where the cytoplasm of the nucleated alveolar epithelial cell becomes attenuated and the border-line between it and the adjacent epithelial cell can clearly be perceived at the end of the extension.

In addition to the above-mentioned observation of nucleated alveolar epithelium, our knowledge of the metabolism of cells and the fact that there have been no observations which positively support the existence of so-called 'non-nucleated plaques' support the belief that covering tissue of alveolar walls is formed of the cytoplasm of nucleated alveolar epithelial cells that attenuates into a long extension. It may be said, therefore, that the frequently discussed existence of the larger, non-nucleated plaques ought to be denied and the alveolar epithelium is composed solely of nucleated cells.

Swigart and his colleague pointed out two types of cells in the interalveolar septa after observing the rat lung with an electron microscope. They are endothelial cells and epitheloid cells, modifications of which are designated septal cell. They recognized the existence of tissue covering the respiratory capillaries and assert that the capillary is not exposed to air space, but they denied the existence of the epithelial covering tissue and the epithelium. According to them, the sole tissue intervening between the air space and the capillary is a continuation of alveolar septal stroma which includes discrete fine-lined fibrillar material and is connected with the central stroma.

The author of this article has obviously perceived the so-called septal stroma which contains such fibrous substances between the epithelial covering and the pulmonary capillary wall. The septal cell, as they call it, is also perceived in this area and it may not be too much to say that their observation has not been adequate, as it has only been performed on rat lungs.

With regard to the epithelial covering tissue, Low has observed in the rat lung that, in the alveolar wall, some cells were by passed by the pulmonary epithelium, the latter continuing uninterruptedly along the surface, and has assumed that the many situations in which the epithelium clearly passes over cells in the alveolar wall without cytoplasmic connection do not exclude the possibility that even these alveolar wall cells may be epithelial, and such a by-passed cell in the epithelial wall which morphologically resembles epithelial cells clearly attenuated in other fields may indeed be an epithelial cell which reaches the surface out of the plane of section. This is beyond the author's understanding, because if such an assumption holds good, it means that the epithelial covering tissue should be two-fold somewhere and such a condition has never been

observed with an electron-microscope by anybody including the author himself. The author of this article believes that the cells situated on the alveolar wall should be considered an non-epithelial when covered with the epithelial covering tissue, even if they morphologically resemble epithelium.

B) *Nucleated Alveolar Epithelium* (figs. 7, 8 and 15)

Except for the cytoplasmic extension, the cell body of the nucleated alveolar epithelium is generally round, oval or cubic and occasionally triangular.

It's position on the alveolar walls does not always seem to be in hollow places of the capillary-net, lumen, as Seemann et al. named "Nischenzellen", nor in the thicker portion of the alveolar walls, as Low asserts. The author has not been able to prove any regularity as to its position.

The nucleus of the epithelium is oval and has some shallow indentations. Sometimes a nucleolus may be observed, depending on the portion selected and cut.

The cytoplasm is generally very finely granular or of irregular mesh and has mitochondria in some places. These are small, round, oval or rod-shaped and comparatively electron dense, and are also perceived in the portion of the epithelial covering which is the extension of the cytoplasm. It remains to be studied and reported whether there is a Golgi apparatus and the so-called endoplasmic reticulum.

C) *The Respiratory Capillary* (figs. 1, 7, 8 and 11)

The respiratory capillary wall is formed of the extended and attenuated cytoplasm of the endothelium and runs with an electron-dense basement membrane along the outer side as in the case of the alveolar epithelium, and therefore the outer line of the capillary wall is clearly seen.

The thickness of the respiratory capillary wall is almost the same as that of the epithelial covering and the cytoplasm of the endothelium often resembles the cytoplasm of the epithelium. The nucleus of the endothelium also often resembles that of the epithelium.

Low has asserted that these cells can be discerned only from the relative position, but according to the author's observation, it can be discerned not only by the position but also by the fact that the internal structure of the endothelial nucleus is comparatively denser than that of the epithelial nucleus and therefore more electron dense, and that the indentation of the nucleus is a little sharper and deeper. Also the cytoplasm of the endothelium is a little denser than that of the epithelium and so they are easily distinguishable electron-density.

D) *Basement Membrane*

In regard to the basement membranes that runs along the bases of both epithelium and endothelium. Low says that they are discretely localized, but the author of this article should like to say that it exists continuously in principle.

The basement membrane is too thin to be observed with a light microscope. When

observed with an electron microscope, it is electron dense and shows a line of uniform size and even quality. Where the epithelial covering tissue and the capillary wall draw quite near without having connective tissue between them, the basement membranes that accompany them often look as though they are completely adherent to each other, but when the entire area is carefully examined, we see that these two basement membranes separate themselves somewhere and show their duality.

The basement membranes of the epithelial covering may be of the same character as those which are at the base of the bronchial epithelium and can be observed with a light microscope, but the author has not observed by electron microscope whether they are continuously connected or not.

E) *The Relation between the Air Space and the Respiratory Capillary* (figs. 1, 2, 7, 9, 10 and 11)

Judging from the above observations, it may be said at least, that the cytoplasm of the endothelium and of the epithelium together with their respective basement membranes, are present between the air in the alveolar space where gas exchange (diffusion) is carried on and the blood in the respiratory capillary, however thin the portion of the tissue between them may be, and accordingly gas exchange is carried on at the place where air and blood come nearest to each other.

Swigart and his colleague, after observing that the endothelial cell is separated from the alveolar wall and that there seemingly is a split between them in certain areas, mention that this physical separation may be due to artifact or to chance. In accordance with the author's observation however, when the capillary is separated from the alveolar wall, there is always the so-called septal stroma composed of fibrous substance between the epithelial covering and the capillary.

It has commonly been observed that one respiratory capillary faces two air spaces that are positioned side by side. The fact was accepted by many scholars who had observed it with a light microscope and Low. But the author can not but be sceptical about the saying of Hayek et al. that the alveolar epithelium is often the same with the respiratory capillary, too. Neither Low nor Swigart allude to this point. According to the author's observation, the serial epitheliums are not common to the two adjacent alveoli, contrary to the assertion of Hayek et al. In such cases, an image of the epithelial coverings that are places back to back and facing the two air spaces is seen, as shown on fig. 6, or the epitheliums that face the two air spaces are seen in back-to-back condition in the same manner with some septal material between them as shown on fig. 7. Judging from this, it may be said that the alveolar epithelium exists separately for each air space, even when the respiratory capillary faces directly two adjacent air spaces.

F) *So-called Septal Stroma*

The space bordered by the electron-dense basement membranes that accompany the epithelium and the endothelium is the structural stratum called the septal stroma.

The thickness of the septal stroma is irregular and includes cell elements and fibrous substances such as lattice-like fibers, collagen fibers and elastic fibers.

It seems there are at least three kinds of cells in the septal stroma. The first cell is comparatively large and has a round or oval nucleus as shown in figs. 11 and 12. Usually the bordering line in such a cell can hardly be seen and it usually is isolated. The character of such a cell is quite unknown at present, but it is often seen situated on the alveolar wall and in such cases, it may not be easy to tell it from the nucleated alveolar epithelium with a light microscope. When carefully observed with an electron microscope, such a septal cell is obviously covered with the epithelial covering tissue, and it is the same cytoplasm as that which is seen in other portion of the septal stroma. Even when it looks like an epithelial cell, it can not be an epithelial cell, as was mentioned above. The character of the septal cell and the problem of whether the nucleated alveolar epithelium has phagocytosis remain to be studied, but the fact that such a cell is often seen may be one reason for the theories that deny the existence of all epithelial cells, assuming them to be histiocytes, and that assert the co-existence of histiocytes with the epithelium.

The second cell that is perceived in the septal stroma is smaller and star-shaped as shown in fig. 13.

The third cell seems to resemble a certain leucocyte as shown in fig. 14 (a part of fig. 3), but its nuclear membrane is thick and distinctly seen and in its cytoplasm there are something electron dense which cannot be considered to be granules. This cell may provably be a so-called clasmatocyte. And here it has been observed to exist free in the structural aperture. The area where it exists is not the capillary lumen but the structural aperture obviously, because the endothelial wall can not be perceived.

G) *Alveolar Wander-cell*

Wander-cell and so-called alveolar dust cell are sometimes found in the air space. Fig. 15 shows a wander-cell found near a part of the alveolar wall. Low said, "It has not been possible to determine with certainty whether or not the epithelial sheet continues uninterruptedly under free cells (alveolar macrophages, etc.) in contact with the surface of the alveolar walls", but in this micrograph there is a continuous epithelial covering.

Fig. 16 shows an alveolar dust cell. Much has been said about their origin, and they will not be discussed at this time.

H) *So-called Alveolar Pores*

Concerning the existence of alveolar pores there are four opinions:

- 1) they are found in normal lungs,
- 2) they are not found in normal lungs,
- 3) they are not found in the lungs of infant animals, but found in adult animals,
- 4) they are the result of a pathologic process.

Miller defined that alveolar pores are minute openings in the walls of the alveoli of

the normal lung, and he said that they do not exist in the normal state, while Hayek accepts their existence. It is necessary to make serial sections to prove their existence histologically, but it is difficult to prove with an electron microscope, as serial observation of sections is almost impossible at present.

Although it is impossible to discuss this problem at present, the following theory may be presented, based on the above-mentioned observations in regard to alveolar structure.

Diffusion may be possible where the septum of, at least, two adjacent air spaces are composed solely of their epithelial coverings, as shown in fig. 6, even if there are no alveolar pores. And again, even when there is some septal stroma between them, it may be possible for the air in an alveolar space to move into the adjacent alveolar spaces through the structural apertures under certain physical and temporal conditions. So-called "Collateral Respiration" may be explained in this way also.

Conclusion

The author has engaged in electron microscopic observation of the alveolar structures since July, 1954, and has arrived at the following conclusion, which concerns chiefly the normal human lung, but it may also apply to lungs of laboratory mammals such as mice, guinea-pigs and dogs except for a few details.

- 1) Membranous epithelial covering tissue covers the entire alveolar walls.
- 2) This epithelial covering tissue of alveolar walls is formed of the attenuated extensions of the cytoplasm of nucleated epithelial cells. Accordingly, only the alveolar epitheliums which are called the nucleated cubic cells are seen on the alveolar wall, and evidence for the existence of non-nucleated plaques does not observe there.
- 3) The respiratory capillary is composed of the attenuated extensions of the cytoplasm of endothelial cells, and each endothelial and epithelial cell has a so-called basement membrane at its base, which separates it from the septal stroma.
- 4) The epithelial cytoplasm and the endothelial cytoplasm with their basement membranes exist between the air in the alveolar spaces where gas exchange takes place and the blood in the respiratory capillary, however thin the structural layer may be.

Each epithelial covering tissue of alveolar walls exists separately for each air space even when a respiratory capillary faces two adjacent air spaces. In other words, there is no alveolar epithelium facing on two air spaces at the same time.

- 5) It is interesting to see various septal cells placed very close to the alveolar wall in the so-called septal stroma containing fibrous substances and cellular elements.

- 6) The origin and history of the alveolar dust cells have not been alluded to in this article. This problem is an important one and must be studied in the future.

- 7) It is very difficult, at present, to prove the existence of the alveolar pores by electron microscope, but it may be surmised, from various observations about alveolar structure, that the diffusing of air between two adjacent alveoli is possible at least

under certain conditions even when there are no alveolar pores. So-called Collateral Respiration may also be explained in this way.

The present author has learned using an electron microscope various new facts about alveolar structures. The fact that scholars arrived at different conclusions and engaged in repeated similar discussions before the electron microscope came into use, even though they studied the same object in the same manner, may be ascribed chiefly to the limited resolving power of the light microscope they employed. The electron microscope has provided a means of solving these problems. But it is not omnipotent; it has its limits also. Careful attention should be paid not only to the objects of the research but also to the systems and methods that should be more broader and more comprehensive.

Acknowledgment

Before ending this brief article, the author wishes to extend his cordial thanks to his director, Prof. Chûzô Nagaishi M. D., who guided him and revised his article and to Assistant Prof. Keinosuke Kobayashi of the Institute for Chemical Research, Kyôto University, who favoured the author with guidance and assistance in electron microscopic observation, and to Naoyuki Nagasawa M. D. of the Surgical Division of the Tuberculosis Research Institute, Kyôto University who gave the author much advice and assistance.

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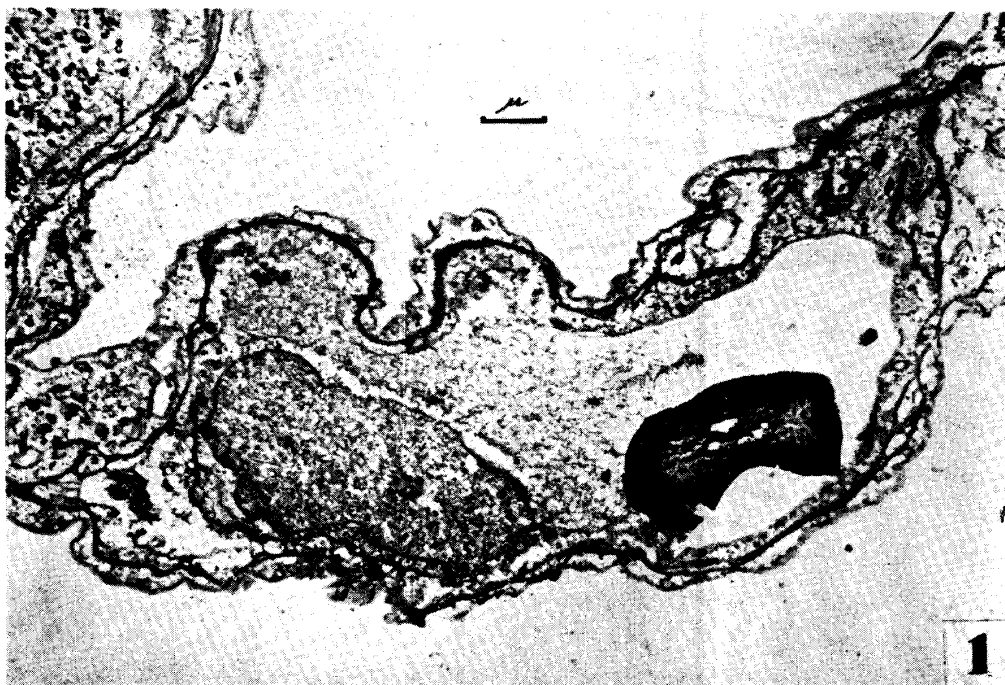
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Explanation of the plates

These plates were reduced in about two-thirds scale of the original size for printing convenience with understanding of the present author (Editor).

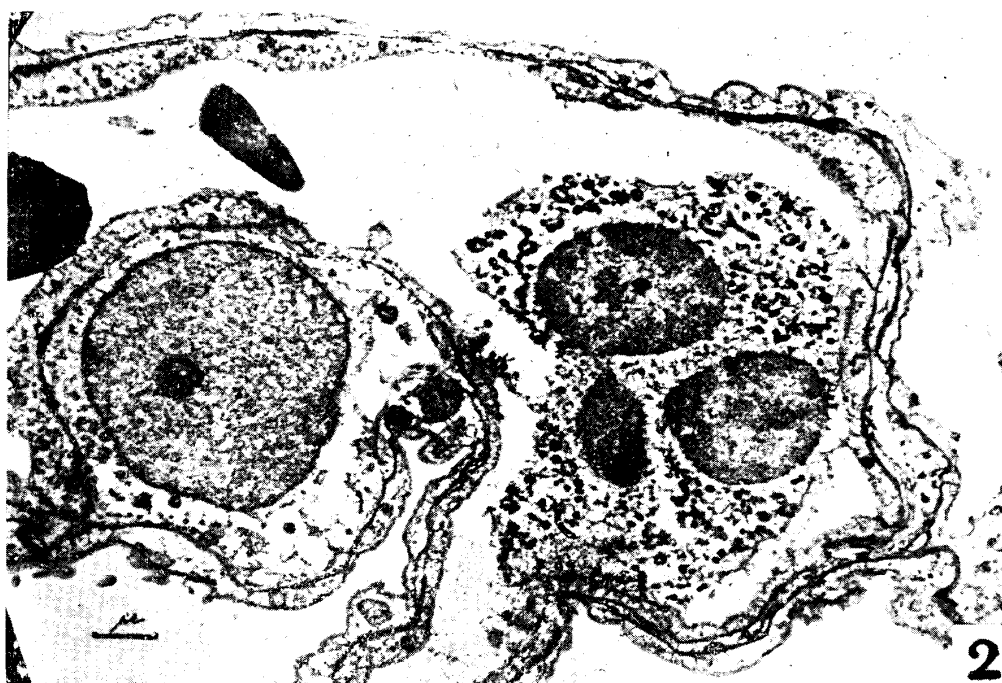
Plates

Bar on electron micrograph is equivalent to 1 micron.



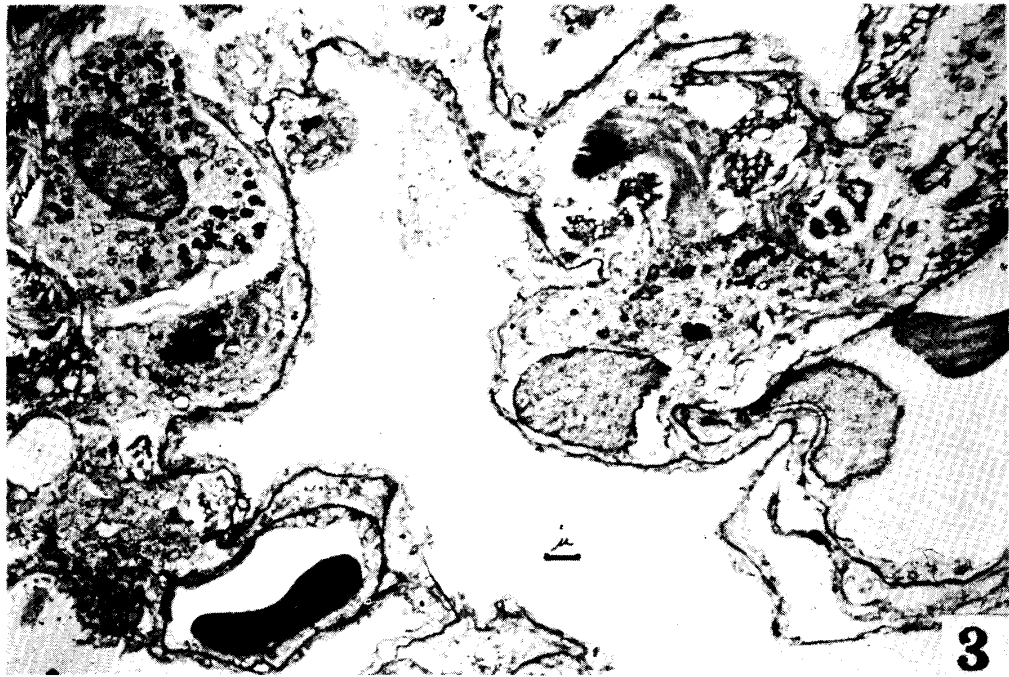
1. Alveolar wall, lung, human ($\times 10000$)

Membranous cytoplasmic covering tissues cover continuously all surfaces facing on air spaces. A endothelium and a erythrocyte are seen.



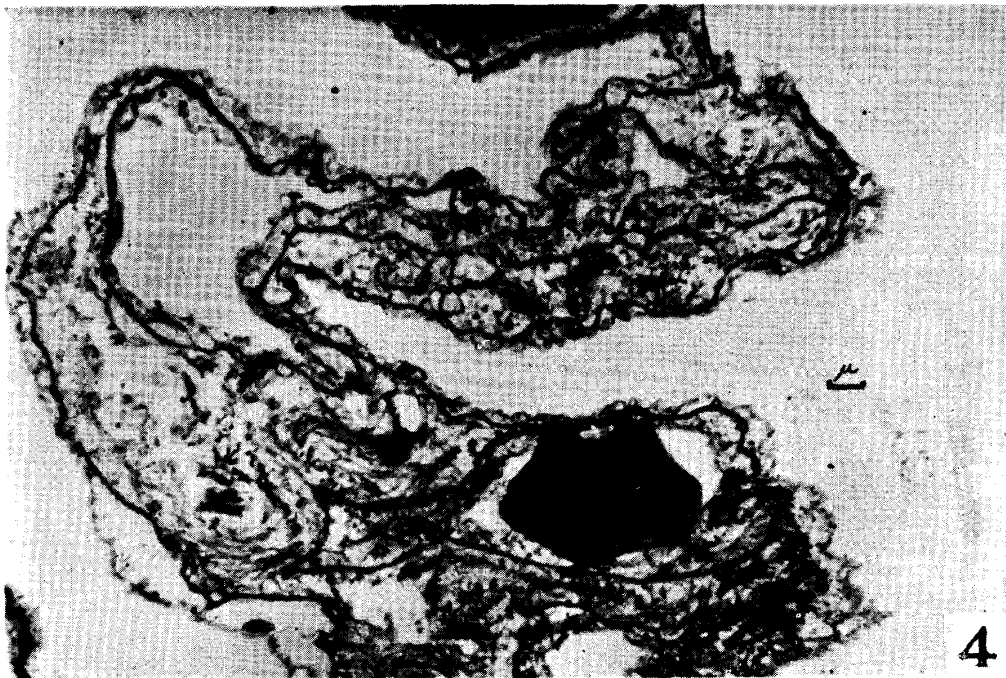
2. Alveolar wall, lung, human ($\times 10000$)

This section is continued from plate 1. Note membranous cytoplasmic tissue covering all surfaces facing on air spaces uninterruptedly. In a capillary, neutrophile leucocyte (3 nuclei) and erythrocytes are seen. Elsewhere a septal cell exists between alveolar covering tissue and capillary wall.



3. Alveolar wall, lung, human ($\times 5600$)

Thin cytoplasmic covering tissues cover continuously all surfaces facing on air spaces. At the upper part of the left side in this micrograph, there is a structural aperture which is filled with a leucocyte. Collagen fibers and elastic fibers are observed in area of septal stroma.



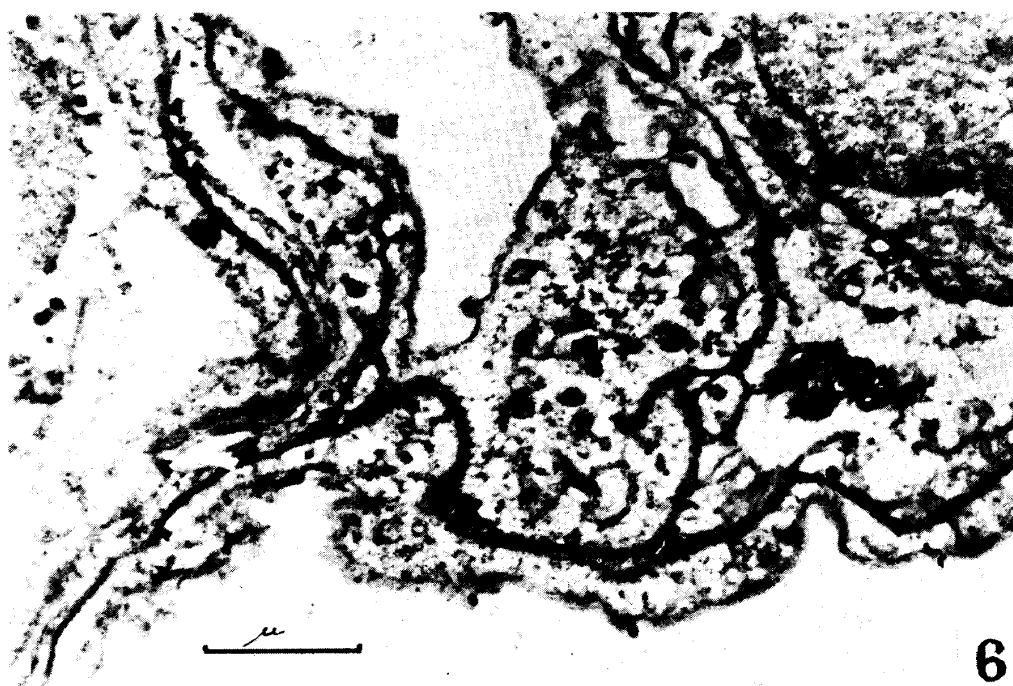
4. Alveolar wall, lung, human ($\times 5500$)

Membranous cytoplasmic covering tissues cover all surfaces facing on air spaces. Two capillaries are recognized in this micrograph, the one being filled with an erythrocyte and the other is vacant. All electron dense lines are so-called basement membrane. In the septal stroma, various fibrous substances are seen.



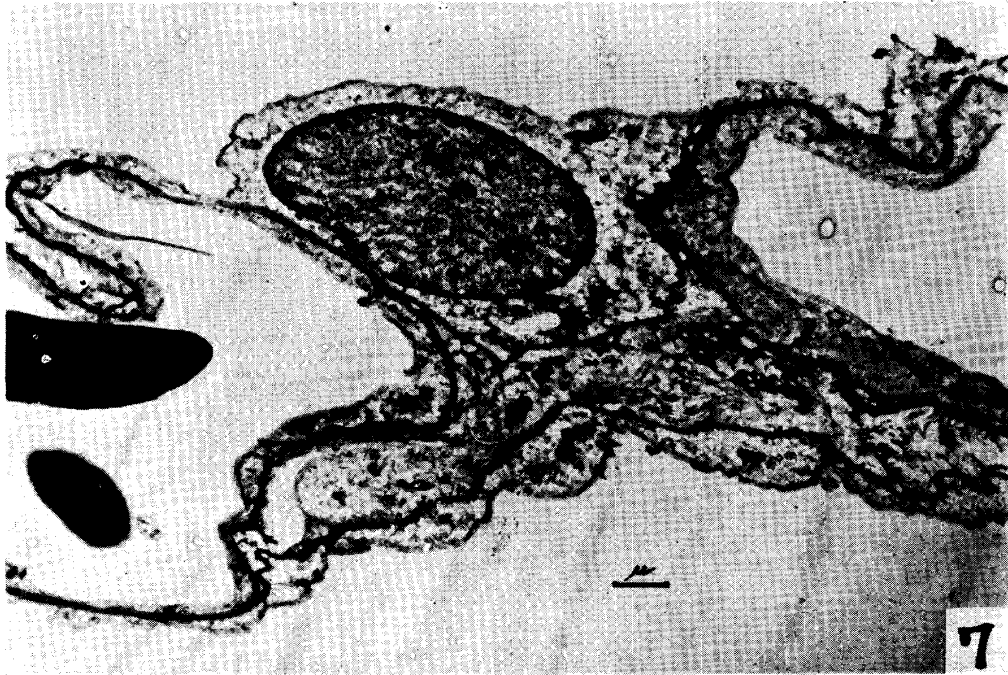
5. A part of alveolar wall, lung, human ($\times 15000$, Cr. shadow)

Attenuated cytoplasmic covering tissue covers surface of alveolar wall that is composed of fibrous material. Heavily osmicated basement membrane draws the line between covering tissue of alveolar wall and stroma.



6. A part of alveolar wall, lung, human ($\times 24000$)

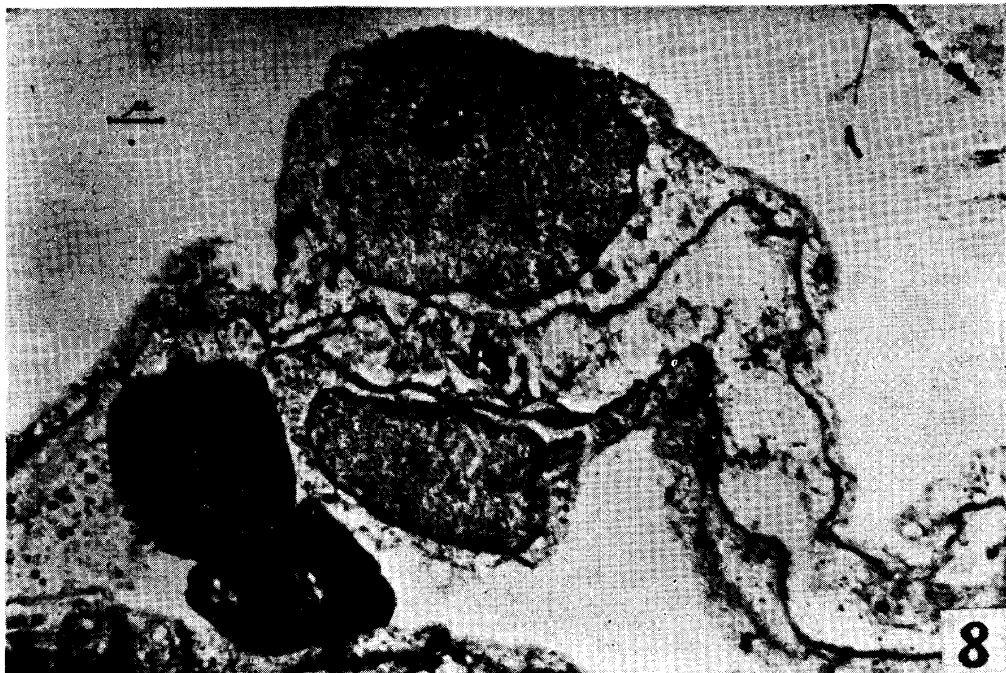
Section showing a part of alveolar wall connecting two adjacent capillaries. The covering tissues facing on two adjoining air spaces are in contact back to back with each other, each accompanying the basement membrane.



7. Nucleated alveolar epithelium, lung, human ($\times 8800$, Cr. shadow)

This epithelium situates two adjacent capillaries. Note cytoplasmic attenuation and extension of epithelium.

Attenuated endothelial cytoplasm is a little electron denser than epithelial cytoplasm in structures. Epithelium and endothelium are accompanied respectively with basement membrane along the base side of them.

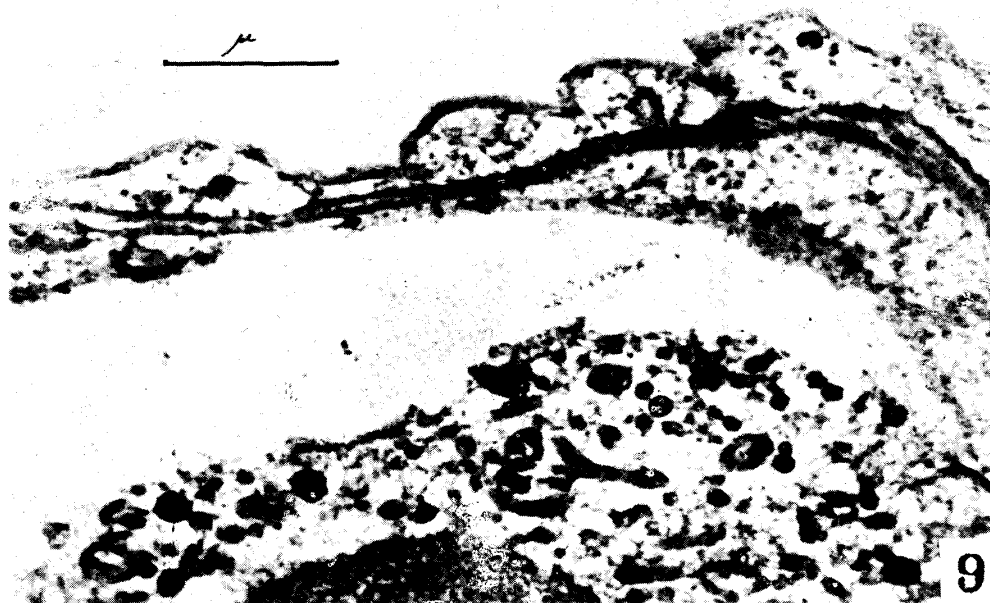


8. Nucleated alveolar epithelium and endothelium, lung, human ($\times 8800$, Cr. shadow)

Note cytoplasmic attenuation and extension of epithelium. A nucleolus is seen in the epithelial nucleus.

Capillary wall is composed of attenuated endothelial cytoplasm.

In this micrograph, epithelium and endothelium are separated with stroma intervening between them, each accompanying basement membrane.



9. A part of alveolar wall, lung, human ($\times 27000$)

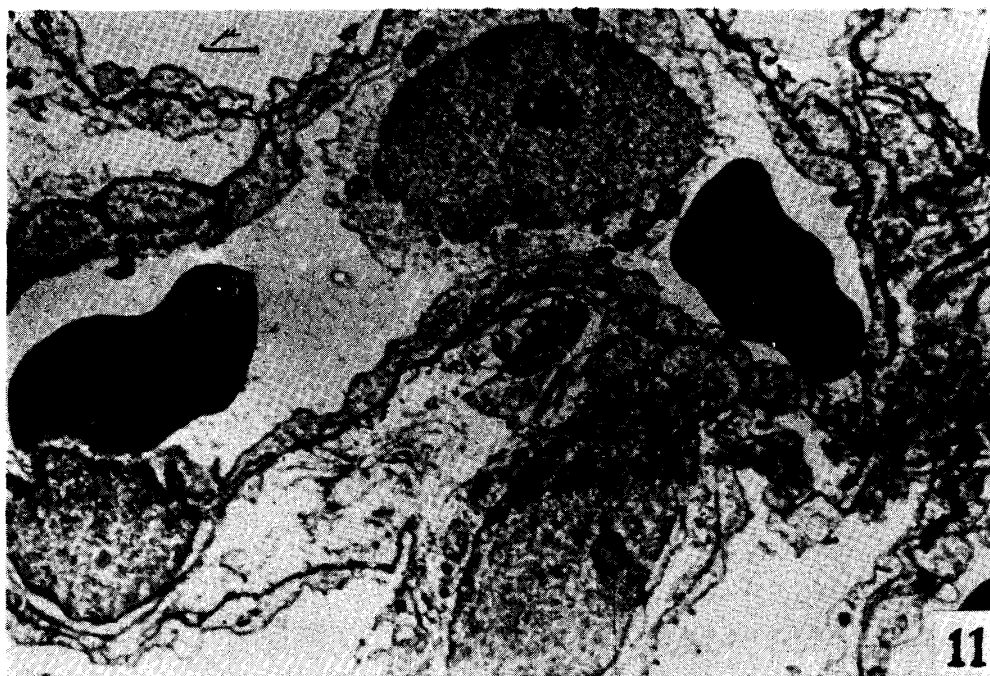
This plate is a part of plate 2.

The upper part in this micrograph is air space and the lower part is capillary. A part of leucocyte is seen in capillary. Cytoplasmic structure of alveolar covering tissue and endothelial cytoplasm, each accompanying with basement membranes, are clearly seen. It seems that gas exchange (diffusion) is carried on at such a place where air and blood come nearest to each other.



10. Alveolar wall, lung, human ($\times 12000$)

Section through a capillary. Note the relationship between the air spaces and the capillary in alveolus where gas-change (diffusion) is carried on. In a capillary, leucocytes are seen.



11. Alveolar wall, lung, human ($\times 9000$)

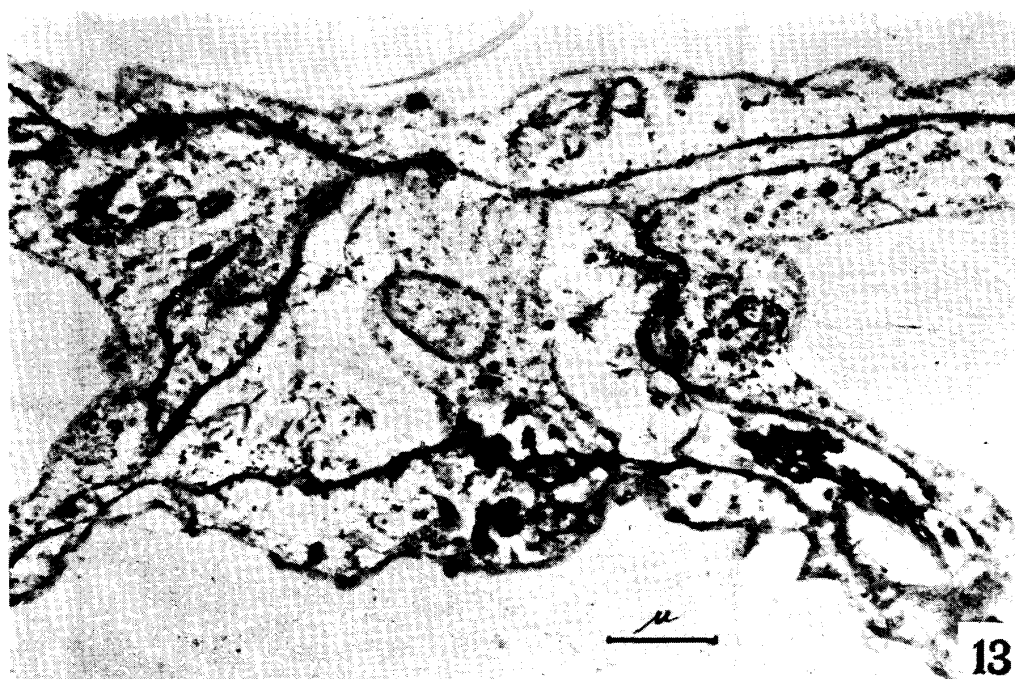
Section through alveolus showing endothelial nucleus and septal cell. In a capillary, a lymphocyte and two erythrocytes are seen.



12. Wall-situated septal cell, lung, human ($\times 14000$)

This plate is a part of plate 2.

This cell is seen wall-situatedly in the alveolar wall. It may not be easy to tell it from alveolar epithelium with a light microscopy. But, in this micrograph, this cell is seen to be covered by attenuated epithelial cytoplasm, and so this is a septal cell undoubtedly. A nucleolus is seen in this nucleus and the cellborder is not so clear.



13. Star-formed septal cell, lung, human ($\times 17000$)

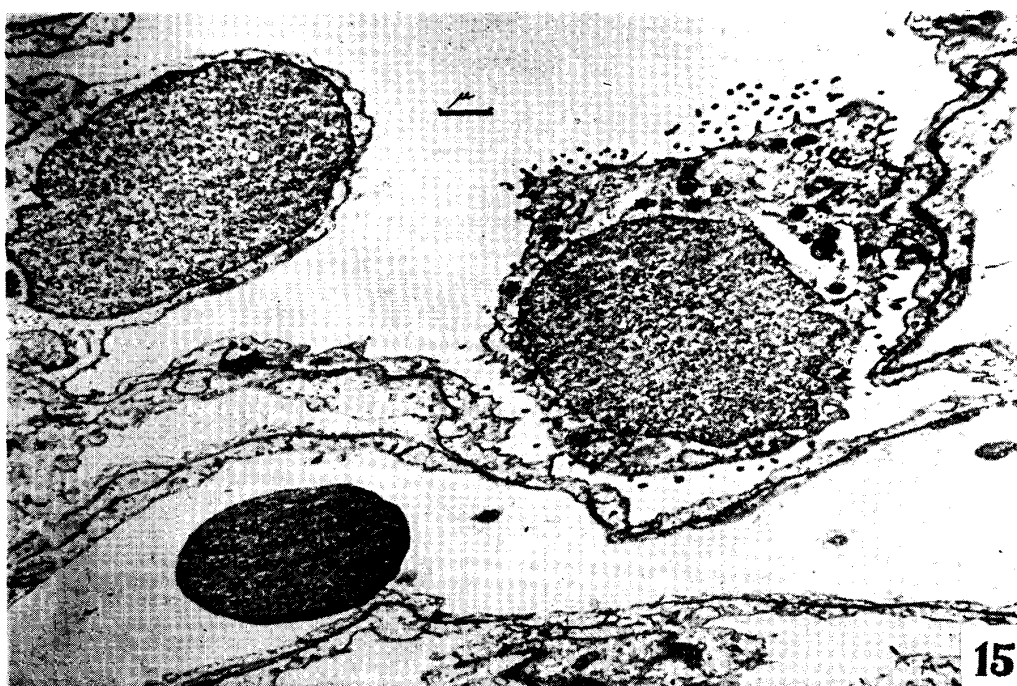
This cell is small shaped and exists at the septal stroma where fibrous material co-exist nearly. The area in which this cell exists is enclosed with two alveolar covering tissue and two capillary walls in this micrograph.



14. A certain septal cell existing at the structural aperture ($\times 11600$, Cr. shadow)

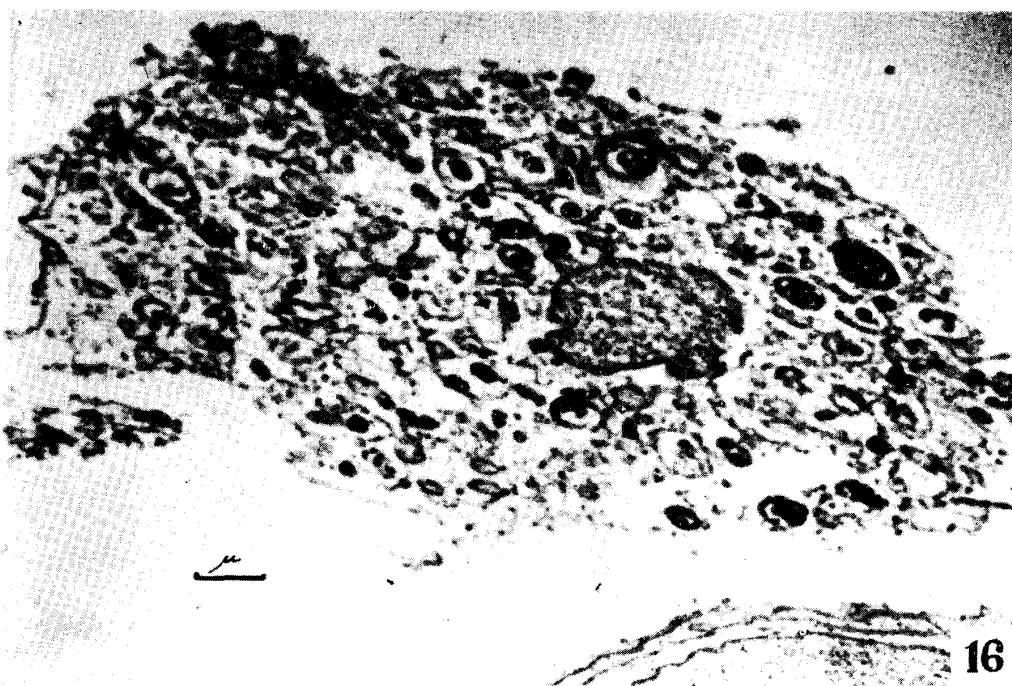
This plate is a part of plate 3.

The cell (perhaps clasmotocyte) fills structural vacant space abutting on the alveolar covering tissue, and a part of its circumference is composed of fibrous material. In cytoplasm of this cell, there are something electron-dense which cannot be considered to be granules.



15. Alveolar wander-cell and alveolar epithelium, lung, human ($\times 9000$)

At the right a wander-cell, at the left a epithelium are seen. Note cytoplasmic attenuation of epithelium. A part of this wander-cell is in contact with alveolar covering tissue which is visible clearly and continuously.



16. Alveolar dust cell, lung, human ($\times 11000$)

Section showing alveolar dust cell free in pulmonary alveolus. Note a small nucleus and characteristic cytoplasm.